Immunobiology (2000) 202, pp. 383–393
© 2000 Urban & Fischer Verlag
http://www.urbanfischer.de/journals/immunobiol

immunobiology

[1]Department of Immunology and [2]Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

A Peptide Based on the CDR1 of a Pathogenic anti-DNA Antibody is more Efficient than its Analogs in Inhibiting Autoreactive T Cells

Eran Eilat[1], Mati Fridkin[2], and Edna Mozes[1]

Received April 18, 2000 · Accepted in revised form September 19, 2000

Abstract

A peptide based on the sequence of the complementarity determining regions 1 (pCDR1) of a pathogenic murine monoclonal anti-DNA antibody (5G12) that bears the 16/6 Id, was synthesized. This peptide was shown to be immunodominant in BALB/c mice, and induced a mild lupus-like disease upon immunization. Furthermore, the pCDR1 when injected in a soluble form was capable of inhibiting the proliferation of lymph node cells primed to either the peptide or the anti-DNA, 16/6 Id antibodies of either murine (5G12) or human (16/6 Id) origin. We have designed and synthesized 39 analogs based on pCDR1 with single amino acid substitutions. Out of the above, two analogs, namely, Asp14 and Ser16 inhibited the proliferative responses of a pCDR1-specific T cell line to its stimulating peptide by more than 50%. These two analogs were therefore further studied. Administration of analog Ser16 concomitant with the immunization with pCDR1 inhibited efficiently the proliferative responses of lymph node cells to pCDR1, although pCDR1 was more efficient in its inhibitory capacity. Neither of the analogs were capable of inhibiting significantly the proliferative responses to the human monoclonal anti-DNA antibody with the 16/6 Id whereas pCDR1 did so efficiently. Thus, pCDR1 is more efficient than all its tested analogs in immunomodulating SLE associated immune responses.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease, which is characterized by the production of autoantibodies to nuclear protein and nucleic acids, accompanied with clinical manifestations (e.g. leukopenia, thrombocytopenia and kidney damage) (1).

The induction of experimental SLE has been previously reported in our laboratory, and was achieved by using the human monoclonal anti-DNA antibody that bears the common idiotype, 16/6 Id (2). The latter has been suggested to play a role in lupus, since it was found in the sera of 54% patients with active disease (3). This antibody could

Abbreviations: APL = altered ligand peptide; CDR = complementarity determining region; Id = idiotype; LNC = lymph node cells; mAb = monoclonal antibody; pCDR1 = peptide based on the CDR1 region; SLE = systemic lupus erythematosus.

0171-2985/00/202/04-383 $ 15.00/0

induce SLE in naive mice of different strains following their immunization (2,4). The injected mice produced high levels of autoantibodies, that include anti-DNA and anti-nuclear protein antibodies, as well as 16/6 Id and anti-16/6 Id-specific antibodies, indicative of the activation of the 16/6 Id network in those mice. Further, clinical manifestations such as: leukopenia, proteinuria and immune complex deposits in kidneys were observed in the immunized mice. Following the latter, it has been demonstrated that experimental SLE can be induced in mice of susceptible strains (e.g. BALB/c, SJL and C3H.SW) by their immunization with either a murine anti-16/6 Id mAb (4) or a murine anti-DNA 16/6^{+} Id mAb, 5G12 (5).

Much evidence supports the central role of T cells in the pathogenesis and development of SLE. BALB/c nude (BALB/c nu/nu) mice that lack T helper function, were resistant to induction of the lupus-like disease (6, 7). Depletion of CD4^{+} T cells was shown to prevent the development of SLE in SLE prone mice (8–10). T cell lines specific for the 16/6 Id were demonstrated to be capable of inducing experimental SLE in syngeneic mice. Furthermore, a 16/6 Id-specific T cell line of C3H.SW origin (H-2^{b}) was capable of inducing experimental SLE in the H-2 compatible C57BL/6 mouse strain (11) that was shown to be resistant to the induction of the disease with the human 16/6 Id antibody (4).

Experimental SLE, although induced in mice that normally do not develop any symptoms of SLE, was found to share features with a murine model for SLE in (NZB×NZW)F1 mice, that develop the disease spontaneously. Thus, BALB/c mice immunized with the human 16/6 Id mAb show similar high levels of anti-DNA antibodies as the lupus prone mice (2). In addition, sequencing of the variable regions coding for the heavy and light chains of anti-DNA mAb isolated from mice afflicted with experimental SLE, show high homology with the variable regions of anti-DNA mAb isolated from (NZB×NZW)F1 mice (13–15).

Since a significant homology was observed between the anti-DNA, 16/6 Id, in experimental SLE and in the SLE prone mice (13) a peptide based on the sequence of the complementarity determining regions 1 (pCDR1) of the pathogenic murine monoclonal anti-DNA antibody (5G12) that bears the 16/6 Id, was synthesized. This peptide was shown to be immunodominant in the BALB/c mouse strains, and induced a mild disease in immunized mice (16). Furthermore pCDR1, when injected in soluble form, was shown to be capable of inhibiting the proliferation of 16/6 Id or 5G12 primed lymph node cells (16).

Despite the fact that pCDR1 showed significant inhibitory effect when injected in an aqueous form, it was of interest to see if analogs to pCDR1 might be more efficient in their inhibitory capacity. Indeed peptides with a single amino acid substitution or altered peptide ligands (APL), were recently extensively studied in various autoimmune diseases as candidates for disease immunomodulation (17–19). Therefore, we prepared a series of analogs to the pCDR1 peptide with single amino acid substitutions and tested their ability to inhibit *in vitro* and *in vivo* autoimmune responses in comparison to the CDR1 based peptide. None of the tested pCDR1 based analogs was more efficient than pCDR1 in inhibiting SLE-associated immune responses.

Materials and Methods

Mice

Inbred BALB/c mice were obtained from Olac, Oxon, UK. Female mice were used at the age of 8–10 weeks.

Synthetic peptides

The CDR based peptide TGYYMQWVKQSPEKSLEWIG (pCDR1) (The CDR is underlined) was prepared with an automated synthesizer (Applied Biosystem model 430A, Germany) using the company's protocols for t-butyloxycarbonyl (BOC) strategy (20). As control, we used a reversed pCDR1, which was synthesized as above.

pCDR1-based analogs were synthesized in the same manner. The following analogs with single amino acid substitutions were prepared:

Methionine at position 5 was replaced by Ala, norleucine
Glutamine at position 6 was replaced by Asn, Asp, Glu and Ala
Tryptophan at position 7 was replaced by Tyr, Phe and Ala
Valine at position 8 was replaced by Trp, Ser and Ala
Lysine at position 9 was replaced by Arg, Glu and Ala
Glutamine at position 10 was replaced by Asp, Glu and Ala
Serine at position 11 was replaced by Asp, Thr and Ala
Proline at position 12 was replaced by His, Ser and Ala
Glutamic acid at position 13 was replaced by Asp, Lys and Ala
Lysine at position 14 was replaced by Glu, Asp and Ala
Serine at position 15 was replaced by Thr, Asp and Ala
Leucine at position 16 was replaced by Ser, and Ala
Glutamic acid at position 17 was replaced by Lys and Ala
Tryptophan at position 18 was replaced by Glu and Ala.

A site directed sequence alteration following a single point alanine residue substitution is widely used with regard to evaluation of key-functional amino acids in biologically active peptides (21). Other substitutions were made in order to evaluate the effect of charge reversal on activity, the importance of hydrophobicity or the possible effect of a polar non-charge residue on activity. Substitution of methionine by norleucin was performed since the latter has a marked effect on the stability of the peptide.

Monoclonal antibodies

The human anti-DNA mAb (IgG1/k) that bears the 16/6 idiotype (16/6 Id) was secreted by hybridoma cells that were grown in culture. The antibody was purified on a protein G-Sepharose column (Pharmacia) (22).

Proliferative responses of T cell lines

T cell lines specific to pCDR1 were established as previously described (11) from lymph node cells (LNC) of BALB/c mice that were immunized with pCDR1. T cells of pCDR1-specific lines (10^4/well) were cultured with 0.5×10^6 irradiated (3000 rad) syngeneic spleen cells in the presence of various concentrations of antigen. Cultures were set in 200 µl enriched medium, containing 10% FCS in flat-bottom microtiter plates (Nunc, Roskilde, Denmark). After 48 hr of incubation at 37°C, [^{3}H]-TdR (0.5 mCi of 5 Ci/mmol) was added. Sixteen hours later, cells were harvested onto a filter paper and radioactivity was measured by a β-counter.

Inhibition of proliferative responses of T cell lines

For inhibition, the pCDR1-specific T cell line was cultured in the presence of pCDR1 (10 μg/well), and analogs at concentrations of 12.5/25/50/100 μg/well.

Inhibition of LNC proliferation

For inhibition of LNC proliferation, 6–8 weeks old mice were injected i.v. with 100 μg of peptide in PBS, concomirant with the immunization with either pCDR1 (10 μg) or the human mAb 16/6 Id (2 μg) in CFA, intradermally in the hind footpads. Ten days following immunization, LNC of immunized mice (0.5×10^6/well) were cultured in the presence of the immunizing antigen. Cultures were set up in 200 μl RPMI 1640 medium supplemented with 2 mM glutamine, non-essential amino acids, 1mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml fungizone, 5×10^{-5}M β-mercaptoethanol and 10 mM HEPES buffer, referred as enriched medium, containing 1% syngeneic normal mouse serum (NMS), in flat 96-well microtiter plates. Following four days incubation, [^{3}H]-thymidine (0.5 mCi of 5 Ci/mmol, Nuclear Research Center, Negev, Israel) was added. Sixteen hours later, cells were harvested and radioactivity was counted.

Percent inhibition was calculated as follows:

$$\%\text{Inhibition} = \left[1 - \frac{\text{DCPM}\cdot\text{proliferation}\cdot\text{with}\cdot\text{analog}}{\text{DCPM}\cdot\text{proliferation}\cdot\text{with}\cdot\text{peptide}}\right]\times 100$$

Results

Activation of a pCDR1 – specific T cell line with single amino acid substituted analogs

We have designed and synthesized 39 analogs based on pCDR1 with single amino acid substitutions. Table 1 demonstrates the pCDR1 peptide and its analogs. These analogs were first examined for their ability to trigger the proliferative responses of a T cell line

Table 1. pCDR1 and analogs

pCDR1: $T_1G_2Y_3Y_4M_5Q_6W/V_8K_9Q_{10}S_{11}P_{12}\ E_{13}\ K_{14}\ S_{15}\ I_{16}\ E_{17}\ W_{18}\ I_{19}\ G_{20}$

Position	Substitution			
5	Ala	Norleucin		
6	Ala	Asp	Glu	Arg
7	Ala	Phe	Tyr	
8	Ala	Trp	Ser	
9	Ala	Arg	Glu	
10	Ala	Asp	Glu	
11	Ala	Asp	Thr	
12	Ala	His	Ser	
13	Ala	Asp	Lys	
14	Ala	Asp	Glu	
15	Ala	Asp	Thr	
16	Ala	Ser		
17	Ala	Lys		
18	Ala	Glu		

Fig
pC
(pC
pro
Res

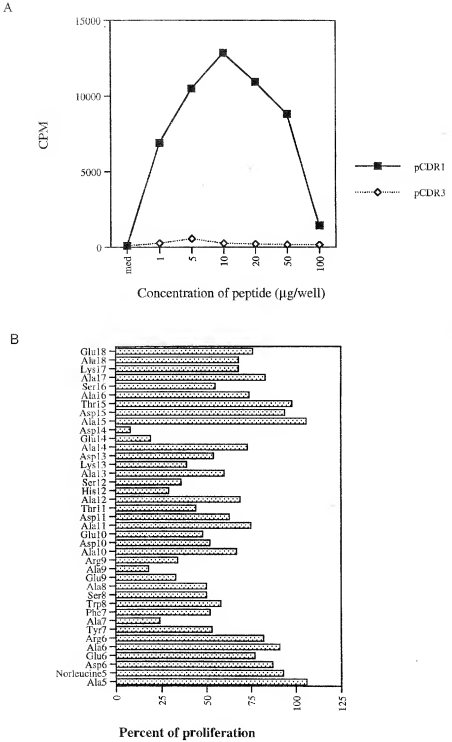

Figure 1. Proliferation of a pCDR1-specific T cell line in the presence of pCDR1 and analogs. A pCDR1-specific T cell line was triggered in the presence of (A) pCDR1 and control peptide (pCDR3)or (B) pCDR1 and its analogs (1–20 μg/well). Results are expressed as percent of maximal proliferative response of analog relative to maximal response to pCDR1 (response to pCDR1=100). Results represent two expetiments.

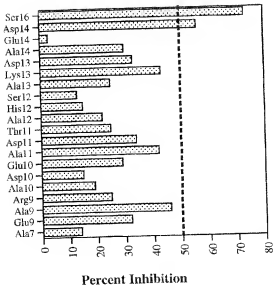

Figure 2. Inhibition of the proliferative response of a pCDR1-specific T cell line by pCDR1 based analogs. A pCDR1-specific T cell line was stimulated with 10 μg/well pCDR1, in the presence of APCs and 100 μg/well of APL. The bars show the percent of inhibition with the specific analog. Results represent two experiments.

specific for pCDR1. Figure 1A demonstrates a representative proliferation assay of the pCDR1-specific T cell line to pCDR1. As can be seen the proliferative response is specific to pCDR1 because a peptide based on CDR3 (pCDR3) of the same mAB did not trigger the pCDR1-specific T cell line to proliferate. Results shown in Figure 1B indicate the proliferative responses of the T cell line to the various analogs. The results are expressed as percentage of the response potential of the line to pCDR1 and demonstrate that some of the peptides triggered a proliferative response similar to that stimulated by the pCDR1, whereas other analogs stimulated minimal or no proliferative responses.

Inhibition of T cell line proliferation

Analogs which did not stimulate the pCDR1 specific T cell line, were further evaluated for their inhibitory effect on proliferation to pCDR1. Figure 2 demonstrates the percentage of inhibition in the presence of 100 μg/well (a concentration that yielded significant inhibition) of analogs, and 10 μg/well of the triggering peptide, pCDR1. As can be seen, two analogs namely Asp14 and Ser16 inhibited the proliferative response of the line to pCDR1 by more than 50%. These two analogs were therefore further studied.

Inhibition of LNC proliferation

It was of interest to further test the ability of analogs Asp14 and Ser16 to inhibit the *in vivo* priming of mice to either pCDR1 or to the whole autoantibody molecule of the human anti-DNA 16/6 Id. To this end, mice were immunized with either pCDR1 or the 16/6 Id in complete Freund's adjuvant and concomitantly injected with pCDR1 or its analogs. Figure 3 demonstrates the results of a representative proliferative experiment

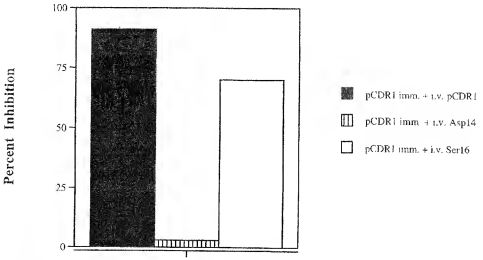

Figure 3. Proliferative responses to pCDR1 in BALB/c mice. Mice were immunized with the pCDR1 in CFA and concomitantly injected i.v. with either pCDR1 or its analogs Asp14 or Ser16 in a dose of 100 μg/mouse. Mice were sacrificed 10 days later and their lymph nodes were stimulated with pCDR1. Results are expressed as percent inhibition of the proliferation to pCDR1 in the immunized and untreated group (CPM= 38646±4020). These results represent two experiments.

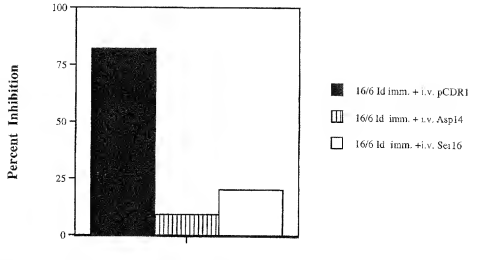

Figure 4. Proliferative responses to 16/6 Id in BALB/c mice. Mice were immunized with 16/6 Id and concomitantly injected i.v. with either pCDR1 or its analogs Asp14 or Ser16 in a dose of 100 μg/mouse. Mice were sacrificed 10 days later and their lymph nodes were stimulated with the immunizing antigen. Results are expressed as percent inhibition of the proliferative response to 16/6 Id in the immunized and untreated group (CPM=8035±760) These results represent two experiments.

in which either pCDR1 or the analogs were injected i.v. in aqueous solution as inhibitors, concomitant with the immunization with pCDR1. As seen in the figure, the injection with analog Ser16 inhibited efficiently (65%) the proliferative response of pCDR1 immunized lymph node cells. It is also shown in the figure that pCDR1 was more efficient in its inhibitory capacity (91%). Analog Asp14 was not capable of down regulating the pCDR1-specific proliferative response.

Figure 4 demonstrates a representative experiment in which pCDR1 and the analogs were tested for their ability to inhibit the *in vivo* priming of T cells to the 16/6 Id. As can be seen neither of the analogs demonstrated significant inhibitory effect on proliferative responses to 16/6 Id whereas pCDR1 inhibited significantly (90%) the proliferative responses to the human 16/6 Id antibody.

Discussion

We have synthesized and tested 39 analogs, with a single amino acid substitution of the CDR based peptide, namely pCDR1. *In vitro* experiments with pCDR1-specific T cell lines and *in vivo* experiments with the pCDR1 and the human monoclonal anti-DNA antibody 16/6 Id, indicated that the pCDR1 was more effective than its analogs in inhibiting SLE associated T cell responses.

The pCDR1 was synthesized based on the complimentarity determining region of a pathogenic monoclonal murine anti-DNA antibody, designated 5G12. This antibody has significant sequences homology with anti-DNA antibodies of (NZBXNZW)F1 mice (14, 15). When injected in soluble form, the pCDR1 inhibits efficiently SLE associated responses including disease induction (16). Because it is of outmost importance to establish a specific, efficient treatment for SLE associated manifestations, we sought out for an analog to the peptide that might be more efficient in inhibiting lupus-like disease.

It has been shown that within the T cell epitopes, some residues act as TCR contact sites and others as MHC contact anchors (19, 23). Therefore, we have attempted the substitution of single animo acids along the pCDR1 molecule (Table 1), in order to address the MHC anchoring residues and the T cell epitope residues (24). We tested the ability of these analogs to proliferate with a pCDR1-specific T cell line. Substitution of amino acids 7–14 had most prominent effect of decreasing proliferative responses, which suggest that this is the region of the T cell epitope, and the MHC anchoring. Analogs that did not trigger proliferative response when incubated with the pCDR1-specific T cell line, were evaluated for their inhibitory effect. Two analogs, namely, Asp14 and Ser16 showed most prominent inhibitory effect of the proliferative response of the pCDR1-specific T cell line, and were further tested. We used these analogs to inhibit *in vivo* priming of mice to either pCDR1 or to the whole autoantibody molecule of the human anti-DNA 16/6 Id. Although analog Ser16 was capable of inhibiting 70% of the ability of pCDR1 primed lymph node cells to proliferate to the latter, its inhibitory effect was much weaker (20%) when lymph nodes were primed with the human anti-DNA 16/6 Id antibody. Overall pCDR1 showed superior inhibitory effect compared to both analogs.

APLs were examined in a few animal models of autoimmune diseases, in attempt to immunomodulate those diseases. The spectrum of action of these peptides ranged from strong to no agonist function with an inverse gradient from strong antagonist to no antagonist function (25). It has been shown that T cell responses associated with *myasthenia gravis* as well as experimental autoimmune myasthenia gravis could be inhibited with the use of analogs to myasthenogenic peptides of the human acetylcholine receptor α-subunit (17, 18, 26). In animal models of experimental autoimmune encephalitis (EAE) the use of APLs altered disease progress. Administration of soluble APL was reported to block development of chronic relapsing EAE (27). In a model for autoimmune insulin-dependent diabetes mellitus, an APL was shown to act as partial TCR agonist (28).

The results of the present study demonstrate that, out of the long list of single amino acid substituted analogs that were prepared and tested, the native CDR1 based peptide has been the most efficient in down regulating SLE associated immune responses. Thus, none of the analogs showed a better *in vivo* inhibitory effect, neither in the case of pCDR1 primed LNC nor when the mice were immunized with the 16/6 Id. These results suggest that the pCDR1 is probably best recognized by the TCR and therefore could be the best candidate for immunomodulation of experimental SLE. This is of great importance since a cumulative line of evidences suggests that the pCDR1 represents a common sequence in murine anti-DNA antibodies isolated from different animal models (14–16, 29). Indeed, results of a series of experiments performed in our laboratory clearly indicate the beneficial effects of pCDR1 in immunomodulating SLE manifestations either in the induced disease with the 16/6 Id (Eilat, Dayan, Zinger and Mozes, unpublished) or in the spontaneous (NZBXNZW)F1 animal model for SLE (30). A significant number of SLE patients demonstrated humoral and cellular responses to pCDR1 (31). Furthermore, pCDR1 was recently shown to be an efficient inhibitor of the proliferative responses of peripheral blood lymphocytes of SLE patients to the human anti-DNA, 16/6Id monoclonal antibody (Sthoeger, Cherniak, Dayan, Elkayam, Segal, Green and Mozes, unpublished). Thus peptide pCDR1 could be considered as a candidate for treating SLE.

Acknowledgement

This study was supported by TEVA Pharmaceutical Industries LTD, Israel.

References

1. Shoenfeld, Y. and E. Mozes. 1990. Pathogenic idiotypes of autoantibodies in autoimmunity. FASEB J., 4: 2646.
2. Mendlovic, S., S. Brocke., Y. Shoenfeld., M. Ben Bassat., A. Meshorer, R. Bakimer and E. Mozes. 1988. Induction of a systemic lupus erythematosus-like disease in mice by a common human anti-DNA idiotype. Proc. Natl. Acad. Sci. USA 85: 2260.
3. Isenberg, D. A., Y. Shoenfeld, M. P. Madaio, J. Rauch, M. Reichlin, B. D. Stollar and R. S. Schwartz. 1984. Anti-DNA antibody idiotypes in systemic lupus erythematosus. Lancet 2: 417.
4. Mendlovic, S., S. Brocke, H. Fricke, Y. Shoenfeld, R. Bakimer and E. Mozes. 1990. The genetic regulation of the induction of experimental SLE. Immunol. 69: 228.
5. Waisman, A., S. Mendelovic, J. P. Ruiz, H. Zinger, A. Meshorer, and E. Mozes. 1993. The role of the 16/6 idiotype network in the induction and manifestation of systemic lupus erythematosus. Int. Immunol. 5: 1293.
6. Mozes, E., S. Brock., H. Fricke, M. Dayan, Y. Shoenfeld and S. Mendlovic. 1989. New experimental autoimmune models. Isr. J. Med. Sci. 25: 692.
7. Mozes, E., S. Mendlovic, F. Kalush, A. Waisman, Y. Shoenfeld and H. Fricke. 1990. Immunoregulation of autoimmune diseases. Isr. J. Med. Sci. 26: 688.
8. Ruiz, P. J., H. Zinger and E. Mozes. 1996. Effect of injection of anti-CD4 and anti-CD8 monoclonal antibodies on the development of experimental systemic lupus erythematosus in mice. Cell Immunol. 167: 30.
9. Theofilopoulos, A. N. and F. J. Dixon. 1985. Murine models of systemic lupus erythematosus. Adv. Immunol. 37: 269.
10. Wofsy, D. 1986. Administration of monoclonal anti-T cell antibodies retards murine lupus in BXSB mice. J. Immunol. 136: 4554.

11. FRICKE, H., S. MENDELOVIC, M. BLANK, Y. SHOENFELD, M. BEN-BASSAT and E. MOZES. 1991. Idiotype specific T-cell lines inducing experimental systemic lupus erythematosus in mice. Immunol. 73: 421.
12. MENDLOVIC, S., H. FRICKE, Y. SHOENFELD and E. MOZES. 1989. The role of anti-idiotypic antibodies in the induction of experimental systemic lupus erythematosus in mice. Eur. J. Immunol. 19: 729.
13. WAISMAN, A. and E. MOZES. 1993. Variable region sequences of autoantibodies from mice with experimental systemic lupus erythematosus. Eur. J. Immunol. 23: 1566.
14. WLOCH, M. K., A. L. ALEXANDER, A. M. N. PIPPEN, D. S. PISETSKY and G. S. G. (1997) Molecular properties of anti-DNA induced in preautoimmune NZB/W mice by immunization with bacterial DNA. J. Immunol. 158: 4500.
15. KATZ, J. B., W. LIMPANASITTHIKUL and B. DIAMOND. 1994. Mutational analysis of an autoantibody: differential binding and pathogenicity. J. Exp. Med. 180: 925.
16. WAISMAN, A., P. J. RUIZ, E. ISRAELI, E. EILAT, S. KONEN WAISMAN, H. ZINGER, M. DAYAN and E. MOZES. 1997. Modulation of murine systemic lupus erythematosus with peptides based on complementarity determining regions of a pathogenic anti-DNA monoclonal antibody. Proc. Natl. Acad. Sci. USA. 94: 4620.
17. KATZ LEVY, Y., M. DAYAN, I. WIRGUIN, M. FRIDKIN, M. SELA M. and E. MOZES (1998) Single amino acid analogs of a myasthenogenic peptide modulate specific T cell responses and prevent the induction of experimental autoimmune myasthenia gravis. J. Neuroimmunol. 85: 78.
18. ZISMAN, E., L. Y. KATZ, M. DAYAN, S. L. KIRSHNER, R. M. PAAS, A. KARNI, O. ABRAMSKY, M. FRIDKIN, M. SELA and E. MOZES. 1996. Peptide analogs to pathogenic epitopes of the human acetylcholine receptor alpha subunit as potential modulators of myasthenia gravis. Proc. Natl. Acad. Sci. USA 93: 4492.
19. SETTE, A., J. ALEXANDER, J. RUPPERT, K. SNOKE, A. FRANCO, G. ISHIOKA and H. M. GREY. 1994. Antigen analogs/MHC complexes as specific T cell receptor antagonists. Annu Rev. Immunol. 12: 413.
20. SCHNOLZER, M., P. F. ALEWOOD and S. B. H. KENT. 1992. *In situ* neutralization in Boc-chemistry solid phase peptide synthesis. Rapid, high yield assembly of difficult sequences. Int. J. Pep. Protein Res. 40: 180.
21. NUTT, R. F., T. M. CICCARONE, S. F. BRADY, C. D. COLTON, W. J. PALEVEDA, T. A. LYLE, T. M. WILLIAMS, D. F. VEBER, A. WALLACE, and R. J., WINQUIST. 1988. In: Peptides: Chemistry and Biology, Proceedings of the 10th American Peptide Symp. G. R., Marshal, Edt., Escom Sci. Publ. Leiden, Netherlands, pp. 444–446.
22. WAISMANN, A., Y. SHOENFELD, M. BLANK, P. J. RUIZ, and E. MOZES. 1995. The pathogenic human monoclonal anti-DNA that induces experimental systemic lupus erythematosus in mice is encoded by a VH4 gene segment. Int. Immunol. 7: 689.
23. JORGENSEN, J. L., P. A. REAY, F. W. EHRICH and M. M. DAVIS. 1992. Molecular components of T-cell recognition. Annu. Rev. Immunol. 10: 835.
24. ROTHBARD, B. J. and R. W. TAYLOR. 1988 A sequence pattern common to T cell epitopes. EMBO J. 7: 93.
25. BACHMANN, M. F. D. E. SPEISER and P. S. OHASHI. 1998. Inhibition of TCR triggering by a spectrum of altered peptide ligand suggested the mechanism for TCR antagonist. Eur. J. Immunol. 28: 3110.
26. KIRSHNER, S. L., E. ZISMAN, M. FRIDKIN, M. SELA and E. MOZES. 1996. Altered peptide ligands of a myasthenogenic epitope as modulators of specific T-cell responses. Scand. J. Immunol. 44: 512.
27. BROCKE, S., K. GIJBELS, M. ALLEGRETTA, I. FERBER, C. PIERCY, T. BLANKENSTEIN, R. MARTIN, N. KARIN, D. MITCHELL, T. VFROMAA, A. WAISMAN, A. GAUR, P. CONLON, N. LING, D. C. WRAITH, A. O'GARRA, C. G. FATHMAN and L. STEINMAN. 1996. Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. Nature 379: 343.
28. GELUK, A., K. E. VAN MEIJGAARDEN, B. O. ROEP and T. H. OTTENHOFF. 1998. Altered peptide ligand of islet autoantigen Imogen 38 inhibit antigen specific T cell reactivity in human type- 1 diabetes. J. Autoimmun. 11: 353.

29. WARD, F. J., J. F. G. KNIES, C. CUNNINGHAM, W. J. HARRIS and N. A. STAINES. 1997. Natural antibodies that react with V-region peptide epitope of DNA- binding antibodies are made by mice with systemic lupus erythematosus as disease develops. Immunol. 92: 354.
30. EILAT, E., H. ZINGER, A. NYSKA and E. MOZES. 2000. Prevention of systemic lupus erythematosus like disease in (NZBXNZW)F1 mice by treating with CDR1 and CDR3 based peptides of a pathogenic autoantibody. J. Clin. Immunol. 20: 268.
31. DAYAN, M., R. SEGAL, A. WAISMAN , N. BROSH, O. ELKAYAM, E. EILAT, M. FRIDKIN and E. MOZES. 2000. Immune response of SLE patients to peptides based on the complementarity determining regions (CDR) of a pathogenic anti-DNA monoclonal antibody. J. Clin. Immunol. 20: 187.

Prof. EDNA MOZES, Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, Tel: 972-8-934-3646, Fax: 972-8-934-4141, e-mail: edna.mozes@weizmann.ac.il